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LETTER TO THE EDITOR

Reply to the comment of Drs Goldman and Vollmer

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Dear Editor,

We appreciate dear Drs Goldman and Vollmer's reaction to our paper [1]. Indeed, the results with HOPE were somewhat surprising to us, and hence, we made every effort to follow the manufacturer's instructions as closely as possible in repeated attempts, before including the results in the manuscript. As detailed in Supplementary file 1 (http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432218/bin/428_2012_1248_MOESM1_ESM.pdf), the fixation process and the deparaffinization procedure were both carried out according to the manual referenced in the reply letter (i.e. the manufacturer's instructions).

Our study investigated the H&E staining morphology and results of IHC assays obtained with tissues that underwent alternative fixation techniques in comparison with the standard formalin fixation, knowing (and that was discussed in the paper) that the stains/assays were originally optimized for formalin-fixed paraffin-embedded tissues. Yet, for HOPE, we not only took care of the correct fixation, dehydration and deparaffinization process as instructed but we also individually optimized the immunohistochemical staining protocol to use, e.g. the recommended citrate buffer (Ventana's CC2 buffer, ref. Supplementary file 3: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3432218/bin/428_2012_1248_MOESM3_ESM.pdf) for antigen unmasking in order to compare the strongest achievable signal for each fixative we tested.

In addition to our results, other investigators also reported histomorphological alterations attributable to the HOPE fixation process, such as "…the HOPE fixed samples generally exhibited a slightly diminished quality of structure. In some of the latter sections, a separation of the epithelium and the underlying lamina propia was observed, additionally, in some cases the epithelium had rolled up. […] After histological staining, HOPE-fixed tissue often also displayed shrinkage artefacts…" [2]. Likewise, "…HOPE fixative led to a loosened tissue structure and a swollen appearance. The H&E process following HOPE fixation did not result in well-stained samples even after optimization of the staining process[…] HOPE fixative dramatically altered the macroscopic appearance of the tissue-engineered constructs. H&E staining of HOPE-fixed constructs also revealed distinct changes in overall tissue structure that rendered them impractical for further morphological analysis." [3]. We remain convinced that we included the HOPE technique into our study in an adequate manner, making sure that the instructions specific for the HOPE process were followed correctly without compromising the overall comparability with other methods. Thus, we still regard the results obtained and presented in the paper as valid.

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